Occurrence of livestock-associated Methicillin-resistant *Staphylococcus aureus* (laMRSA) in domestic pigs and wild boars in Germany

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Abstract

In order to identify differences in the frequency of MRSA in conventional pig herds compared to organic pig herds, and wild boars in Germany, three different studies on the occurrence of MRSA were conducted. The results show a considerably higher amount of MRSA-positive pigs in conventional herds than in pigs of organic herds and in wild boars (no MRSA were found in the tested wild boars). All MRSA isolates belong to the clonal line MRSA ST398, which is known as livestock-associated MRSA (laMRSA). Our two hypotheses for the considerably higher occurrence of MRSA in conventional pig production are: a) the circulation of this special clonal line laMRSA ST398 between conventional pig herds and b) better MRSA transmission conditions because of a higher pig density in conventional pig production systems than in organic pig pig production systems or in the wild boar population.

Introduction

The detection of the so called Methicillin-resistant *Staphylcoccus aureus* strains (MRSA) that are multidrug resistant, especially against β-lactam antimicrobials, through a penicillin binding protein has been known in human medicine since the 60's (BARBER, 1964). Initially, the monitoring of MRSA was limited to hospital infections. Over the following decades, MRSA strains were more and more often found in the regular population (TIEMERSMA et al., 2004), which resulted in the differentiation between hospital-acquired MRSA (haMRSA) and community-acquired MRSA (caMRSA) (NAIMI et al., 2002). The variability of the MRSA prevalence in Europe is wide and shows a spread of 1% in the Netherlands to more than 30% in several other European countries (TIEMERSMA et al., 2004).

Animals as sources for caMRSA were identified for the first time by the detection of MRSA as causative agent of mastitis in a cow herd in Belgium (DEVRIESE, 1972). Afterwards, an increasing number of publications about sporadic cases of MRSA in other domestic animals such as dogs, cats (LOEFFLER et al., 2005), and horses (CUNY et al., 2006) were released. Since 2005, there have been reports from the Netherlands about a special clonal MRSA line in pig herds, the Multilocus Sequence Typing type ST398 (VOSS et al., 2005; DE NEELING et al., 2007). Accordingly, MEEMKEN et al. (2008) detected a comparable high frequency of the occurrence of MRSA in German pig herds. All pig isolates from the study in Germany were assigned to the MLST type ST398. Although the reports from several countries about MRSA in pigs show that ST398 is almost the only clonal MRSA line to be found in porcine populations, it is not justified to assume that this is a clonal line that is exclusively adapted to pigs, since several data show that ST398 has been isolated from other mammalian species including humans, both as latent colonizer and as cause of severe infections (WITTE et al., 1997). These facts demonstrate that the MRSA complex has a zoonotic component, which is in the case of ST398 closely linked to food animal populations: apart from pigs, there are also reports on calves and cows (WAGENAAR, 2008) and poultry (NEMATI et al., 2008). Therefore, the term livestock-associated MRSA (laMRSA) has been newly established. The following investigations are a contribution to the knowledge on the occurrence of MRSA in different pig populations and husbandry systems.

Material and Methods

In three different studies investigations on the occurrence of MRSA in different pig populations with different husbandry systems were carried out as follows:

a) MRSA as nasal colonizer of domestic pigs from conventional husbandry systems. Therefore we tested 687 pigs from 347 different farms and age groups, which were sent to the Field Station for Epidemiology for clarification of herd health problems by necropsies and laboratory diagnostics. In none of these cases, the shown clinical diseases were associated with *Staphylococcus aureus*. Before dissecting the pigs, nasal swabs were taken, sent to the National Reference Centre for Staphylococcus of the Robert Koch-Institute, Germany, and cultured on MRSA-selective chromagar plates. From all MRSA isolates the MLST-type was determined.
b) MRSA in dust samples taken in 6 organic pig husbandry systems and in 7 conventional husbandry systems. Conform to the European Food Standard Agency's approach of the survey on the prevalence of Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs (SANCO/3100/2007 – Rev.2), we collected 5 dust samples (500 cm^2 each) per herd, pooled, enriched and cultured them in our laboratory.
c) MRSA as nasal colonizer of wild boars hunted in different parts of Germany. From 75 hunted wild boars nasal swabs were taken and cultured on MRSA-selective chromagar plates.

Results

In study a) from the 678 tested pigs in 85 cases MRSA (a frequency of 13%) could be found as nasal colonizer. The MRSA isolates all were identified as belonging to the livestock-associated MRSA type ST398. At the herd level the frequency of positive herds was 18% (62 positive herds). Table 1 demonstrates the results according to the different age groups.

Tab. 1: MRSA positive pigs according to the age groups

Age group (weight class)	**No. of pigs (%)**	**MRSA ST398 positive (%)**
Suckling pigs (1,4 kg – 8 kg)	197 (29%)	20 (10%)
Weaning pigs (> 8 kg – 25 kg)	252 (37%)	44 (17%)
Grow-finishers (> 25 kg – 40 kg)	88 (13%)	12 (14%)
Finishers (> 40 kg – 110 kg)	102 (15%)	9 (9%)
Productive sows (> 110 – 291 kg)	39 (6%)	0 (0%)
Total No. of animals	**678**	**85 (13%)**

The highest frequency of positive results with almost 15% was in the group of the weaners and in the group of the grow-finishers. Also in 10% of suckling pigs and finishers MRSA could be isolated. None of the 39 breeding sows were tested positive for MRSA. This may be due to the small number of tested sows. As shown in Tab. 2 the rate of positive herds steadily increased with the number of pigs submitted and tested per herd. Only 7% of the herds with one submitted pig were positive, 20% of the herds with 2 pigs, 25% of the herds with 3 pigs, and even 50% of the herds with 4 submitted pigs were positive.

Tab. 2: Rate of MRSA positive tested herds according to the number of tested pigs

No. of tested pigs per herd	**No. of herds**	**No. of positive tested herds (%)**
1 tested pig	151	11 (7%)
2 tested pigs	118	24 (20%)
3 tested pigs	47	12 (25%)
4 tested pigs	20	10 (50%)
> 4 tested pigs	11	6 (55%)
Total No. of tested herds	**347**	**63 (18%)**

b) Current results of the dust samples of organic pig farms show a considerably lower prevalence of MRSA as compared to the samples of the tested conventional herds (Tab. 3).

Tab. 3: Results of testing dust samples from organic vs. conventional husbandry systems

	No. of tested herds	**MRSA positive herds (%)**
"organic" dust samples	6	1 (16%)
"conventional" dust samples	7	7 (100%)

c) All 75 tested nasal swabs from wild boars were MRSA-negative. Besides other bacteria like Streptococcus ssp., Methicillin-sensitive Staphylococcus aureus (MSSA) was detected in 10 wild boars.

Discussion

In accordance with several similar studies in other countries (DE NEELING et al., 2007; WAGENAAR, 2008; WITTE et al., 2007), our results on the MRSA frequency in pigs (nasal colonization) at the individual pig level seem to be the same. However, the measured herd prevalence of 18% from pigs raised in conventional husbandry system seems to be highly underestimated (cf. Table 2), because only few animals per herd were tested. And yet, the more pigs per herd were tested the more MRSA positive herds (one positive pig and more) could be ascertained. The true herd prevalence, therefore, must be supposed to be far above 50%.

Overall the results indicate that the MRSA load in conventional husbandry systems (in the nasal cavity and in dust samples) is higher than in organic husbandry systems and even much higher than in the population of wild boars. The latter cannot be attributed to a natural non-susceptibility of wild boar for *Staphylococcus aureus*, since 10 wild boar were colonized with MSSA. Assumptions for the higher MRSA detection rate in conventional husbandry systems than in organic herds are the higher animal density in conventional herds (i.e. smaller distances between the animals, better transmission conditions), the commonly more frequent use of antibiotics, and the more frequent contacts (circulation of MRSA between herds) with other conventional herds through animal movements than in organic pig herds.

Conclusions

More detailed research into the risk factors of MRSA is needed. Presently however, it is not justified to assume a direct link between the use of antibiotics at a specific farm and the occurrence of MRSA, especially considering that different strategies of antibiotic treatments were applied, but always the same clonal line of MRSA was found.

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